

H. MAHESWARI DEVI* & S. V. KRISHNA*: **Embryology of
Sagina procumbens L. and *Drymaria cordata*
Willd. (Caryophyllaceae)**

H. マヘシュワリ デビ*・S. V. クリシュナ*: アライドツメクサと
ヤンバルハコベ (ナデシコ科) の胚学的研究

Tulasne (1855) was the first to study the embryogeny and early developmental stages of a few genera of the subfamily Diantheae of Caryophyllaceae. The embryological work in this family is reviewed by Schnarf and Davis up to 1931 and 1966 respectively. Subsequent contributions include those of Guignard (1965, 1966a, b), Vignon & Lebegue (1965, 1966), Longevialle (1967), Petria (1971), Pal & Murthy (1972, 1974), Newcomb & Fowke (1973), Werner & Zenktele (1973), Pal (1974), Ramji (1975), Chaudhary & Narayana (1978), Kamelina & Yakovleva (1982) and Singh & Taneja (1985).

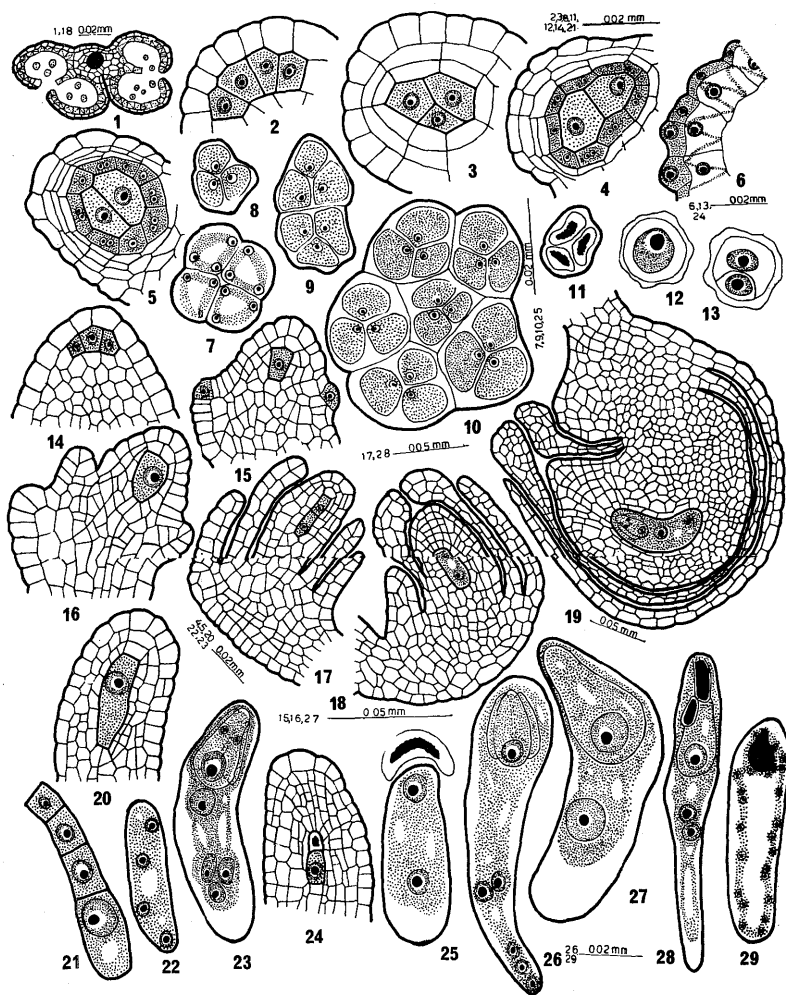
Although the development of the female gametophyte, endosperm and embryo received substantial attention, practically very little work has been done with regards to anther and male gametophyte. Therefore, a detailed life history of *Sagina procumbens* and *Drymaria cordata* is investigated in the present study.

Material and methods The material collected from the hills of Darjeeling is fixed in formalin-acetic-alcohol. Dehydration, infiltration and embedding in paraffin wax are done in customary methods (Johansen 1940). The section thickness was maintained between 9 and 13 μ and the sections are stained in Delafield's haematoxylin.

Observations *Sagina procumbens* is an annual herb while *Drymaria cordata* is a glabrous shrub with swollen nodes. The opposite leaves are petiolate and inflorescence is characteristic cincinnus type.

Microsporogenesis. The anther is dithecous and tetrasporangiate (Fig. 1) with Dicotyledonous type of wall. It comprises the endothecium, middle layer and tapetum besides the epidermis (Figs. 2, 3, 4, 5). However, the middle layer at the pollen mother cell stage undergoes periclinal divisions resulting in two

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Figs. 1-29. 1. Transverse section of tetrasporangiate anther showing pollen grains. Note. Fibrous thickenings in endothecium. 2-5. Transverse section of anther lobes showing development of wall layers and sporogenous tissue. 6. Endothecium and persistent epidermis of anther. 7. Pollen mother cells in meiosis. 8. Tetrahedral pollen tetrad. 9, 10. Agglutinated pollen tetrads. 11. Degenerating pollen tetrad. 12, 13. 1 and 2-celled pollen grains respectively. 14-19. Development of ovule. 20. Mature megaspore mother cell. 21. Linear megaspore tetrad. 22-23. 4 and 8-nucleate embryo sacs respectively. 24. Longitudinal section of ovule with functional dyad. 25, 26. 2 and 8 nucleate embryo sacs respectively. 27. Fertilized embryo sac. 28. Embryo sac showing zygote and 2 endosperm nuclei. Note. degenerating pollen tube and synergid. 29. Embryo and nuclear endosperm.

layers of cells (Fig. 5). The tapetum is secretory, monomorphic and uniseriate with binucleate cells (Fig. 5). The two middle layers are ephemeral. The endothecium attains fibrous thickenings and forms the fibrous endothecium (Fig. 6). The epidermis remains persistent even after the dehiscence of the anther.

The hypodermal archesporium consists of a plate of 3 or 4 cells in transection (Fig. 2) and each row in longitudinal section is 4 or 5 celled. The primary sporogenous cells undergo a few mitotic divisions and form the pollen mother cell. Prior to reduction division the pollen mother cells neither round off themselves and separate from each other nor do their walls get thickened. Instead, they remain agglutinated forming a mass. The reduction division in all the cells of the pollen mother cells are synchronous. Simultaneous cytokinesis in them results in the formation of pollen tetrads (Figs. 7, 9, 10). They are both tetrahedral and decussate in *S. procumbens* and only tetrahedral in *D. cordata* (Fig. 8). Although the tetrads remain agglutinated the young microspores within the tetrad become separated from each other. The mature pollen grain is two celled at the time of shedding (Figs. 12, 13).

In *D. cordata* pollen degeneration at various stages of development is quite common (Fig. 11). In a tetrad one, two or three or all the four spores become degenerated. The pollen grains also degenerate either at one or two nucleate stage.

Megasporangium. The ovary in *S. procumbens* is superior, tricarpeal, syncarpous and trilocular with two rows of ovules on axile placentation while in *D. cordata* it is unilocular with four rows of ovules on free central placentation. The ovule is bitegmic, crassinucellate and campylotropous (Fig. 19). The ovular primordium which consists of a homogeneous mass of meristematic cells is trizonate with dermal, subdermal and central zones (Fig. 14). Initiation of ovular curvature occurs after the differentiation of the hypodermal archesporium. The initials of the inner integument become differentiated by the time the megaspore mother cell is formed while those of outer are differentiated by the time the megaspore mother cell is ready for reduction division (Figs. 15, 16). Both the integuments are dermal in origin and two layered thick. However, the tip of the inner integument at the 2-nucleate embryo sac stage becomes multilayered. Further, the inner integument overgrows the outer and alone contributes to the micropyle (Figs. 17-19).

At the megaspore mother cell stage a few nucellar epidermal cells on the

sides undergo periclinal divisions and result in the formation of 2 layered nucellar epidermis. The primary parietal cell that is cut off from the archesporium undergoes periclinal divisions and forms 2 or 3 layered parietal tissue. Divisions in the parietal tissue and subdermal cells of the young nucellus result in the production of massive nucellus. These dermal and subdermal files of cells are arranged in a fan shaped pattern and the nucellus forms a cap at the micropylar side. By the time an 8-nucleate embryo sac is formed due to excessive unilateral growth of the nucellus at the chalazal end, the ovule curves and becomes campylotropy. The nucellus is however, absorbed by the growing embryo sac and in a mature seed 4 to 6 layers persist as perisperm.

Megasporogenesis and megagametophyte. The female hypodermal archesporium in *D. cordata* is single celled (Fig. 15) and is 2 or 3-celled in *S. procumbens* (Fig. 14). In the latter usually one or less frequently two of the cells and in the former a single cell develops further and cut-off a parietal cell towards outside and megaspore mother cell towards inside (Fig. 15, 16).

The development of the female gametophyte in the two species differs from each other and hence separate account is given to each.

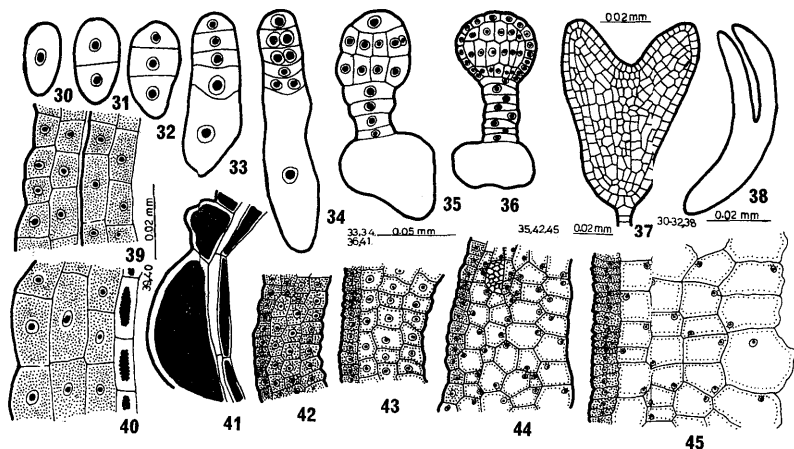
In *S. procumbens* the megaspore mother cell undergoes reduction division producing a linear tetrad of megaspores. The chalazal one is functional and undergoes three mitotic divisions resulting in a monosporic, *Polygonum* type of 8-nucleate embryo sac (Figs. 20-23).

In *D. cordata* the megaspore mother cell undergoes first meiotic division and produces a dyad of cells. The upper cell of the dyad degenerates while the lower undergoes three free nuclear divisions and produces an 8-nucleate embryo sac of Bisporic, *Allium* type (Figs. 24-26).

In both the cases synergids are hooked. The three antipodal cells are ephemeral.

Embryo sac degeneration is common in *D. cordata*.

Fertilization, Endosperm and Embryo. Fertilization is porogamous. Endosperm is *ab initio* nuclear. The primary endosperm nucleus undergoes free nuclear divisions resulting in the formation of a number of nuclei which are distributed uniformly at the periphery of the embryo sac enclosing a large central vacuole (Figs. 27-29). Cellularization commences at about the globular embryo stage. It proceeds from the micropylar to towards chalazal end of the embryo sac ultimately filling the entire embryo sac with cellular tissue.



Figs. 30-45. 30-38. Successive stages in the development of embryo. Note. Vesicle in Figs. 34, 36. 39-41 stages in development of seed coat. 42-45. Fruit wall development.

The zygote is clavate in shape. It undergoes a transverse division resulting in a terminal cell *ca* and a basal cell *cb* (Figs. 30, 31). The basal cell *cb* does not divide further, but enlarges and remains as a vesicular cell without taking any part in the development of the embryo proper (Figs. 33-36). The terminal cell *ca* divides transversely into two cells *cc* and *cd* (Fig. 32). The cell *cd* undergoes a transverse division and gives to cells *m* and *ci*. The cell *cc* divides transversely into *l* and *l'*. The three cells *l*, *l'* and *m* divide vertically resulting in two juxtaposed cells in each tier. Meanwhile the cell *ci* divides transversely and two superposed cells *n* and *n'* are formed. The latter undergoes one more transverse division producing *o* and *p*. Thus a six tiered linear proembryo is formed from the terminal cell *ca*.

The tiers *l*, *l'* and *m*, undergo two more vertical divisions at right angles to each other resulting in octants in each tier.

Further vertical and transverse divisions in the young embryo lead to the development of globular and heart shaped embryo.

From the above it is evident that the embryo proper and also the suspensor are derived from the terminal cell *ca* alone, while the basal cell *cb* remains as a large vesicular cell. This is characteristic of *Sagina* variation of Caryophyllad type.

Seed coat. In the initial stages of ovule both outer and inner integuments are two layered (Fig. 40). As already mentioned, at 2-nucleate embryo sac stage the inner integument becomes 3 or 4 celled thick at the micropylar region. After fertilization the inner layer of the inner integument degenerates (Fig. 40). The two layers of the outer integument remain healthy. Thus, the mature seed coat comprises an outer epidermis of the inner integument and the two layers of the outer integument. The seed coat is three layered and non-multiplicative. At about globular embryo stage the epidermal cells of the testa and tegmen develop abundant tannin (Fig. 41).

Fruit wall. The ovary wall from the archesporial cell stage to mature embryo sac stage is 4-celled thick (Figs. 42, 43). After fertilization ovary wall becomes 5 or 6 layered. By the time the mature embryo is formed the cells of the outer epidermis become radially elongated and accumulate abundant cytoplasm whereas the cells of the inner epidermis become very much enlarged and show vacuolation. The cells in between the two epidermal layers become somewhat enlarged. All these layers together constitute the fruit wall (Figs. 44, 45).

Conclusion The present observations and those made earlier (Cook 1903, Gibbs 1907, Perotti 1913, Soueges 1922, 1924, 1925, Recen 1927, Joshi 1936a, b, Devine 1950, Buell 1952a, b, Pal 1953, Guignard 1963, 1965, 1966, 1967, Pritchard 1964a, b, Petria 1971, Pal & Murthy 1972, 1974, Werner & Zenkteler 1973, Pal 1974, Ramji 1975, Chaudhary & Narayana 1978, Kamalina & Yakovleva 1982, Singh & Taneja 1985) reveal that the members of Caryophyllaceae are characterised by the following embryological features: anther is tetrasporangiate with mono- or dicotyledonous type of wall development, tapetum is secretory with two nucleate cells; pollen tetrads are tetrahedral, isobilateral, decussate; pollen grains are 2- and 3-celled and 3-colpate; ovule is hemianatropous to campylotropous, bitegmic and crassinucellate; parietal tissue is well developed; nucellar epidermis at places divides periclinally resulting in two or three layers of cells; perisperm present; micropyle is formed by the inner integument; embryo sac development is of *Polygonum* or *Allium* or *Adoxa* type; endosperm is nuclear and embryo is of Caryophyllad or Solanad type.

In all these embryological features the family Caryophyllaceae although is sharing kinship with the rest of the families of the centrospermalian plexes it differs in certain morphological and embryological characters such as cincinnus type of inflorescence, superior, two to five carpellary and 1 to 5 locular ovary,

different types of placentation; capsular fruit, mono- or dicotyledons type of anther wall development; glandular tapetum with 2-nucleate cells, *Polygonum* or *Allium* or *Adoxa* type of embryo sac development and Caryophyllad type of embryogeny.

Within the family certain variations occur. The anther wall although is of dicotyledonous type in general (Davis 1966) in *Stellaria media* (Pal 1953), *S. aquatica* (Pal 1974), *Sagina procumbens* and *D. cordata* (present data) it shows variation in the presence of more than one middle layer.

Stellaria media (Joshi 1936a), *S. procumbens* and *D. cordata* (present study) show an interesting feature in the agglutination of pollen mother cells and occurrence of compound tetrads appearing like pollinia/massula of families like Mimosae, Asclepiadaceae and Orchidaceae.

In *Polycarpon loeflingiae* (Pal 1953) *Stellaria media* (Moliono 1959) and in *S. procumbens* (present report) the ovary is superior and trilocular with axile placentation, whereas in *Dianthus chinensis* (Buell 1952) and *Drymaria cordata* (present data) the ovary tricarpeal and unilocular with free central placentation.

In the family Caryophyllaceae, caryophyllad type of embryo development is characteristic. However, in *P. loeflingiae* Pal (1953) reported Solanad type of embryo development.

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アライドツメクサ *Sagina procumbens* L. と ヤンバルハコベ *Drymaria cordata* Willd. の花粉形成, 胚嚢, 胚乳, 胚形成を報告した。アライドツメクサの胚嚢形成は *Polygonum* type であるのに, ヤンバルハコベは *Allium* type であった。それ以外は他のナデシコ科のもの同様で, 胚乳形成は nuclear, 胚形成は *Caryophyllad* type である。